

Use of DAPI Cytofluorometric Analysis of Cellular DNA Content to Differentiate Spitz Nevus from Malignant Melanoma

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Cellular DNA content was measured for the purpose of differentiating Spitz nevus from malignant melanoma using the cytofluorometric technique. DNA was stained by 4', 6-diamidino-2-phenylindole, and measured by microfluorometer. Among 20 Spitz nevi examined, 18 of them showed a diploid DNA distribution histographic pattern similar to that of acquired pigmented nevi. The other two Spitz nevi had a few polyploid cells with the major population of cells containing diploid DNA content. In contrast, all malignant melanomas showed an aneuploid DNA distribution histographic pat-

tern. The DNA index values of cells from Spitz nevi distributed in the similar range to that of acquired pigmented nevi and separated from those of malignant melanomas distributed in a much higher range.

Our results suggest that cytofluorometric analysis of cellular DNA content reflects the biologic behavior more sensitively than do conventional clinical or histologic criteria, and that it serves as a useful aid for the differentiation of Spitz nevus from malignant melanoma. *J Invest Dermatol* 95:154-157, 1990

Spitz nevus behaves biologically as a benign neoplasm despite its resemblance to malignant melanoma in clinical and histologic features [1,2]. It is one of the most difficult diagnostic problems in the field of pigmentary neoplasms, although the differentiation of Spitz nevus from malignant melanoma is critically important. Toward this purpose several authors proposed histologic criteria for both conditions [3-6]. These criteria have been useful and satisfied many clinical demands for the diagnosis; however, many cases still remain to be clearly differentiated. Measurement of the nuclear area and the feulgen stained-DNA content using image-analysis cytometry has recently been reported as an aid to differentiation [7]. Although this combined method enabled some differences between Spitz nevus and malignant melanoma to be indicated, it needs further study to determine if it can be developed into a useful adjunctive technique in the diagnosis of melanocytic lesions.

We have previously reported that microfluorometric analysis of DNA content using 4', 6-diamidino-2-phenylindole (DAPI) is useful for detecting premalignant or malignant conditions of the skin [8,9]. Therefore, we attempted to discriminate Spitz nevus from malignant melanoma using this method and report here on the successful results of our study.

MATERIALS AND METHODS

Patients and Materials Twenty excised specimens of Spitz nevus (age, 1-85 years; mean age, 20 years), and 20 specimens of malignant melanoma (age, 21-79 years; mean age, 48 years) were

studied. Twenty specimens of acquired pigmented nevus (compound or intradermal type) (age, 4-60 years; mean age, 32 years) were also examined as controls of benign melanocytic tumor. The histologic features enabled Spitz nevus to be divided into 13 cases of "spindle-cell-predominate type," six cases of "epithelioid-cell-predominate type," and one case of "mixed type." Specimens of malignant melanoma were all from the nodules of a case of nodular, eight cases of superficial spreading, 10 cases of acral lentiginous, and one case of unclassified melanoma. Acquired pigmented nevi were usually confirmed by the patients' clinical histories, and giant pigmented nevi larger than 1.5 cm in diameter were excluded from our study to prevent unexpected contamination of congenital nevi [10].

All the materials for the DNA measurement were prepared from the central portion of nevi or tumors in order to eliminate topographic differences in comparison among specimens.

Measurement of Cellular DNA Content The measurement of cellular DNA content was performed by the methods previously reported [8]. Briefly, paraffin-embedded specimens were cut into units 50 μ m thick and deparaffinized. Simultaneously, thin sections of 4 μ m were cut and observed after hematoxylin-eosin stain, firstly to verify the histologic characters and secondly to identify the area for evaluation. The central portion of the tumor masses was trimmed and then loosened by rinsing in Ringer-Lock's solution containing 0.05% collagenase (type IV, Sigma) for a day at 37°C. The loosened specimens were ultrasonified (Sonifier 185E, Branson, Danbury, Connecticut) to obtain cell suspension. The isolated cells were then washed with salt solution, transferred to glass slides, and fixed with 100% methanol. Nuclear DNA was then stained with 4', 6-diamidino-2-phenylindole (DAPI) and observed with an Olympus-MMSP-RFS microfluorometer (Olympus, Tokyo, Japan). The intensity of fluorescence was recorded and analyzed using a personal computer combined with the microfluorometer. One hundred to 300 intact cell nuclei were observed and measured to obtain a DNA distribution histogram from a single specimen.

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Abbreviation:

DAPI: 4', 6-diamidino-2-phenylindole

Stromal lymphocytes were used as controls for the normal diploid DNA content.

Analysis of DNA Distribution Histogram Polyploid cells were defined as those having a DNA content exceeding 3 times the diploid DNA content. The aneuploid cell population was defined on DNA distribution histogram as an apparent peak that differed more than 10% from the nearest euploid peak. Using the criteria described above, the DNA histogram pattern was classified into five types:

- 1) Diploid pattern
 - a) s-diploid pattern: a single apparent fraction of cells containing diploid DNA content.
 - b) p-diploid pattern: a major fraction of cells with diploid DNA content accompanying a small fraction of cells of either or both hyperdiploid and/or tetraploid DNA content.
- 2) Polyplod pattern: diploid pattern accompanying a small number of polyplod cells.
- 3) Aneuploid pattern
 - a) s-aneuploid pattern: at least one apparent aneuploid cell fraction.
 - b) p-aneuploid pattern: diffuse distribution of cells with various amounts of DNA content.

In order to evaluate DNA ploidy as a simple value, the DNA index was calculated by dividing the mean value of the DNA content of all the tumor cells measured in a specimen by the value of the diploid DNA content of the same specimen. The Student *t* test was used for the statistical analysis of DNA index values among comparative groups.

RESULTS

Representative examples of DNA distribution histograms of Spitz nevi, malignant melanomas, and acquired pigmented nevi are shown in Figs 1–3. Eighteen specimens of 20 Spitz nevi revealed a

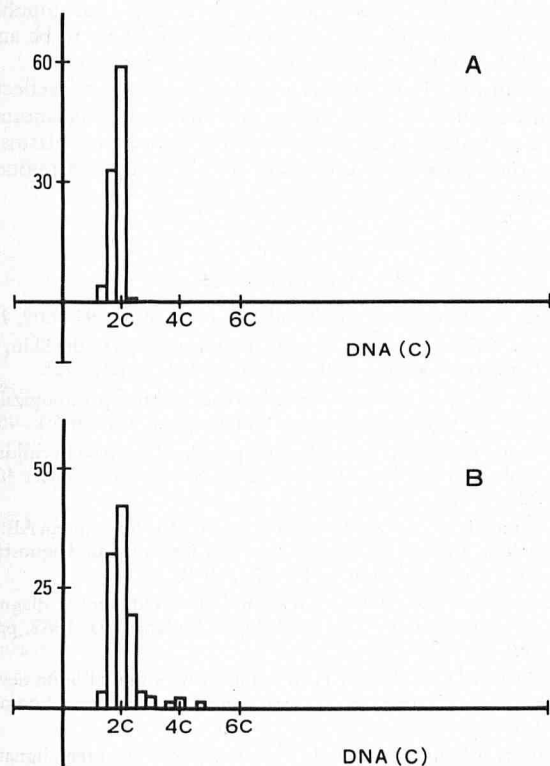


Figure 1. Representative DNA distribution histograms of Spitz nevi. Abscissa, DNA content (DNA(C)); ordinate, percentage of the number of cells measured at the given DNA content. A and B are s-diploid, p-diploid patterns, respectively.

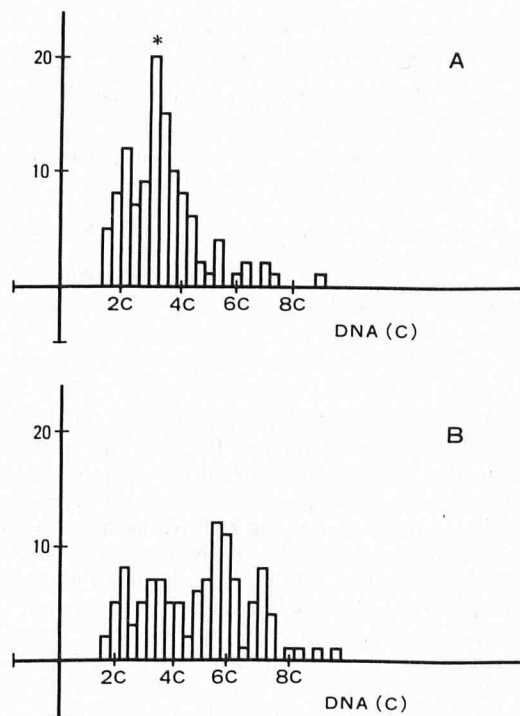


Figure 2. Representative DNA distribution histograms of malignant melanomas. Asterisk indicates an aneuploid cell fraction. A and B are s-aneuploid and p-aneuploid patterns, respectively.

diploid pattern, two of which showed only a single fraction of cells with diploid DNA content (s-diploid pattern, Fig 1A), and the other 16 accompanied a small fraction of cells with hyperdiploid and/or tetraploid DNA content (p-diploid pattern, Fig 1B) (Table I). Two Spitz nevi had a small number of polyplod cells with a

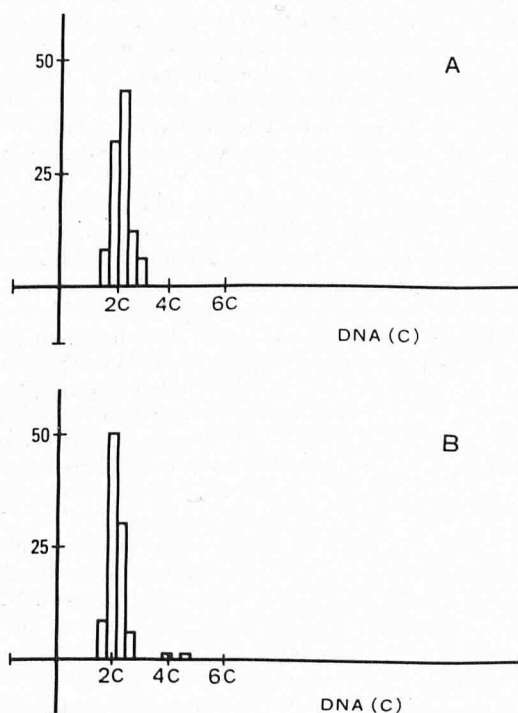


Figure 3. Representative DNA distribution histograms of acquired pigmented nevi. A and B are s-diploid and p-diploid patterns, respectively.

Table I. Histographic Pattern and DNA Index of Spitz Nevus, Malignant Melanoma, and Acquired Pigmented Nevus

	Histographic Pattern (number)	DNA Index, Mean \pm SEM (range)
Spitz Nevus	s-diploid (2)	1.09 \pm 0.07 (1.01–1.29)
	p-diploid (16)	
	Polyploid (2)	
Malignant Melanoma	s-aneuploid (16)	1.82 \pm 0.35 (1.34–2.50)
	p-aneuploid (4)	
Acquired Pigmented Nevus	s-diploid (3)	1.09 \pm 0.04 (1.02–1.16)
	p-diploid (17)	

major diploid cell fraction (polyploid pattern) (Table I). No apparent aneuploid cell fraction could be found in any Spitz nevi. The aneuploid pattern was a feature for all the malignant melanomas examined, 16 of which had one or more apparent fractions of aneuploid cells (s-aneuploid pattern, Fig 2A) and the other four showed a diffuse distribution of cells with various amounts of DNA content (p-aneuploid pattern, Fig 2B) (Table I). Acquired pigmented nevi did not have any abnormal polyploid cells or aneuploid cell fractions, and histographically showed a diploid pattern, three of which were s-diploid (Fig 3A), with the other seventeen being p-diploid (Fig 3B) (Table I). Thus, Spitz nevus is fundamentally similar to acquired pigmented nevus and clearly different from malignant melanoma in terms of its DNA distribution histographic pattern.

The DNA index values of Spitz nevi (1.01–1.29; mean value \pm SEM, 1.09 \pm 0.07) distributed in the range similar to that of acquired pigmented nevus (1.02–1.16; mean value \pm SEM, 1.09 \pm 0.04) (Table I). The DNA index values of malignant melanomas (1.34–2.50; the mean value \pm SEM, 1.82 \pm 0.35) were significantly higher than those of Spitz nevi ($p < 0.001$) (Table I). Furthermore, no overlapping of DNA index values was notable between Spitz nevi and malignant melanomas.

DISCUSSION

Because both Spitz nevus and malignant melanoma nodule are characterized histopathologically as dermal aggregates of cytologically atypical melanocytes, differentiation of Spitz nevus from malignant melanoma can be difficult and even impossible in some cases by conventional criteria [2,3]. In the present study, the DNA content of cells from Spitz nevi, malignant melanomas, and acquired pigmented nevi were measured for two purposes, firstly to overcome the problem of diagnosis, and secondly to ascertain the biologic character of Spitz nevus.

All the Spitz nevi were histographically diploid pattern or diploid pattern with a few polyploid cells (polyploid pattern), which clearly differed from the aneuploid pattern of malignant melanomas. DNA index values are also clearly different between Spitz nevi and malignant melanomas. Although a Spitz nevus presented a relatively high DNA index value (1.29), it was still lower than the lowest one of the melanomas (1.34). Our results suggest that DAPI-DNA cytofluorometry provides us with a useful aid for differentiating Spitz nevus from malignant melanoma. Spitz nevi presented a similar DNA histographic pattern and DNA index as the acquired pigmented nevi examined in our study. This evidence is likely to provide a cytologic basis for the current concept that Spitz nevus is a condition of pigmented nevi [1,2,3,6].

All of the malignant melanoma specimens showed DNA aneuploidy in our study. This finding is fundamentally compatible with other investigations except for the high frequency of DNA aneuploidy [11,12]. There are two possible explanations. Firstly, our materials were all nodules of nodular, superficial spreading, or acral lentiginous melanoma. Because the nodule is regarded as highly malignant in comparison with the macular lesion of melanoma [13], a high frequency of DNA aneuploidy is reasonably expected. In fact, DNA aneuploidy appeared at a much lower frequency in the macular lesion (radial growth phase) of melanoma [14]. Secondly,

our methods were different from those of other investigations (flow cytometry) and provided more precise information, as described later.

Although DNA aneuploidy has been found to be a characteristic of malignancy in several organ systems [15–17], it remains conflicting if it corresponds to pigmentary neoplasms. Recent flow-cytometric studies [11,12] reported that one of 34, or four of 16 histologically benign nevi show DNA aneuploidy as compared to other investigations that reported only a diploid pattern [15,18]. Our study demonstrated a diploid pattern in all the acquired pigmented nevi examined and confirmed of latter reports. The following differences in materials and methods probably provide the explanation for our results. We excluded from our study congenital nevi, especially giant ones, to prevent the contamination of nevi having a premalignant character and frequently showing DNA aneuploidy [18]. Our DAPI-DNA cytofluorometry, which has been improved for quantitative cytofluorometry [19], enabled us under microscope to distinguish a single, morphologically intact nucleus for measuring its fluorescence intensity. This method thereby is advantageous in minimizing the problems of many multinucleated cells, which sometimes appear in the histology of nevi [20], the breakdown of cellular nuclei during material preparation, and cytoplasmic melanin pigment [21], all of which disturb the DNA ploidy determination by flow cytometry. Serially sectioned, hematoxylin-eosin-stained histology also serves to realize what part of tumors we can actually observe and measure.

Our study demonstrated that some Spitz nevi and acquired pigmented nevi had a number of cells containing hyperdiploid and/or tetraploid DNA content. They may be S and G₂/M phase cells of the cell cycle, respectively, which reflects a proliferative activity of those nevi. A small number of polyploid cells, however, were detected in only two cases of Spitz nevi. DNA polyploidy, especially the occurrence of a great number of polyploid cells, is usually a feature of malignant or premalignant tumors reflecting the mitotic irregularity [8,16]. In contrast, a small number of polyploid cells sometimes appear in rapidly growing benign neoplasms like irritated seborrheic keratoses or growing viral wart (our unpublished results). These polyploid cells, therefore, are likely to be another feature of the high proliferating potential of Spitz nevus.

In conclusion, DAPI-DNA cytofluorometry well reflects the biologic behavior of Spitz nevus and malignant melanoma and clearly discriminates between the two pigmentary neoplasms. This method thus provides a useful aid for the diagnosis pigmentary neoplasms.

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